N-TERMINAL SEQUENCES OF THE α AND β SUBUNITS OF THE LECTIN FROM THE GARDEN PEA (*PISUM SATIVUM*)

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1. Introduction

Lectins constitute a group of proteins mainly found in the seeds of a wide variety of plants and in certain invertebrates. These proteins have proven to be useful tools for the study of protein–carbohydrate interactions and the topography of cell surfaces. When immobilised on Sepharose, lectins may be used for the fractionation of cells and for the isolation of glycoproteins and cell receptors.

Certain lectins are able to induce mitogenesis in lymphocytes a phenomenon analogous to the stimulation of immune cells by a specific antigen [1–3].

Although lectins have been extensively studied for their biological properties, few structural data are available to relate their activity to their structure, except for Concanavalin A [4]. In this paper we describe the subunit structure and N-terminal sequences of the α- and β-subunits of the phytohemagglutinin of the garden pea (*Pisum sativum*), a protein with sugar binding properties similar to those of concanavalin A.

2. Materials and methods

Pea-lectin was purified from the seeds of the garden pea (*Pisum sativum*), variety Wonder van Kelvedon) by a slight modification of the method of Trowbridge [5]. Sephadex G-75 instead of G-100 was used as affinity adsorbent. The gels for polyacrylamide gel electrophoresis in the presence of SDS were made as described by Laemmli [6] using the running buffer of Weber and Osborn [7]. Polyacrylamide gel electrophoresis in the presence of urea was performed according to Panyin [8].

Sequence analysis was done with a Beckman 890C Sequenator using a 0.1 M Quadrol program [9]. The phenylthiohydantoin derivatives were identified by gas chromatography [10], thin layer chromatography [11] and amino acid analysis on a single column Durrum 500D analyser, after back-hydrolysis in HI at 150°C for 24 h.

3. Results and discussion

3.1. *Separation and molecular weight of the subunits*

Pea-lectin is known to consist of two types of subunits, held together by noncovalent bonds [5,12]. These two subunits differ by their molecular weight and are designed as α-subunit (the small one) and β-subunit (the large one). The molecular weight of the subunits was estimated from their migration in SDS electrophoresis as 7000–8000 for the α-subunit and 17 000–18 000 for the β-subunit. These results are in agreement with the data obtained by Trowbridge [5]. A molecular weight of 10 500 for the α-chain was reported by Marik et al. [12]. The α- and β-chains were separated by gel filtration on Sephadex G-75 in 6 M guanidine–HCl (fig.1). In these conditions, however, the β-subunit was eluted from the column in the same position as bovine serum albumin (mol. wt 68 000), while the α-subunit emerged from the column at essentially the same volume as lysozyme (mol. wt 134 000). These results suggest that aggregation of both chains occurs even in 6 M guanidine–HCl. The β-chains form at least tetramers (mol. wt 68 000), while the α-chains form dimers (mol. wt 14 000).

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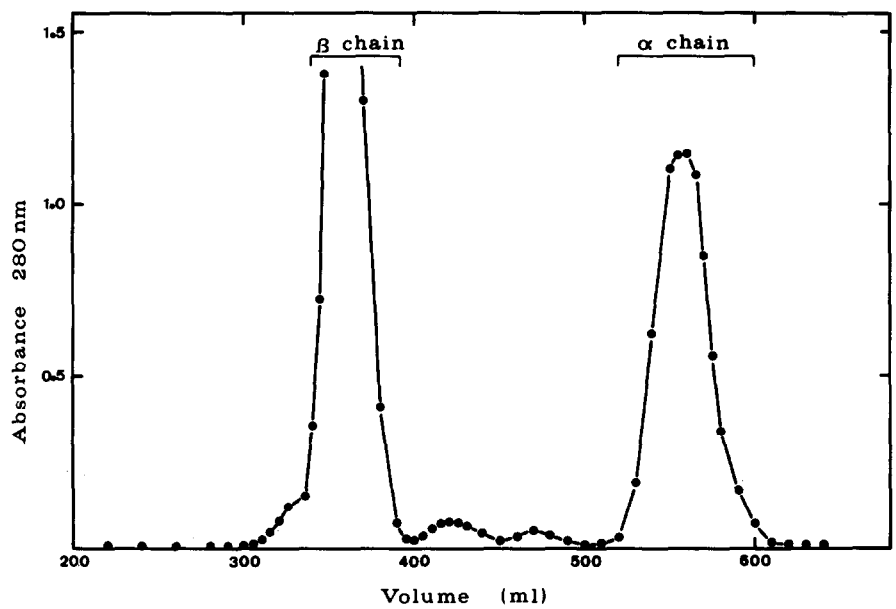

Fig.1. Separation of the α- and β-subunits of pea-lectin on Sephadex G-75 (3 X 200 cm) in 6 M guanidine–HCl. Fractions of 5 ml were collected and pooled as indicated.

3.2. *Sequence data*

The amino terminal sequences of the two subunits were determined by automated Edman degradation (fig.2). A comparison with Concanavalin A [4] yielded a striking homology of residues 13–29 of the α-chain with sequence 80–99 in Con A. The sequence of an octapeptide which is identical to positions 14–21 from our α-chain was reported recently for a pea lectin heavy chain peptide [13].

3.3. *Existence of iso-lectins*

When subjected to polyacrylamide gel electrophoresis in 6 M urea pH 3.2, the α-chains migrated as two sharp bands, suggesting the presence of two

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                1                                   10                                  20
α-chain pea-lectin: Val Thr (Ser) Tyr Thr Leu (Ser) Asp Val Val (Ser) Leu Lys Asp Val Val Pro Glu Trp Val
Concanavalin A  : Ala ———— [      ] Val —— Tyr Asp —— Asp —— Asn ——— Leu ————————
                72                                  80

                21                                  30                                  38
                Arg Ile Gly Phe (   ) Ala Thr Thr Gly Ala Glx Tyr Ala Ala (   ) Glx Gly Leu
                — Val — Leu Ser —— Ser ——— Leu Tyr Lys Glu Thr Asn Thr Leu Leu
                                                    99

                1                                   10                                  20
β-chain pea-lectin: Thr Glu Thr Thr Ser Phe Leu Ile Thr Lys Phe Leu Pro Asp Gln Gln Asx Leu Ile Phe
                :

                21
Fig.2. Sequence data.   Glx Gly Asx Gly Tyr Thr
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Fig.2. Amino terminal sequences of the α- and β-chains from *Pisum sativum* as compared to Con A. Homologies are indicated by a solid line. Parentheses ( ) correspond to unidentified residues or residues identified by only one method. Deletions [ ] were introduced to maximize homology.

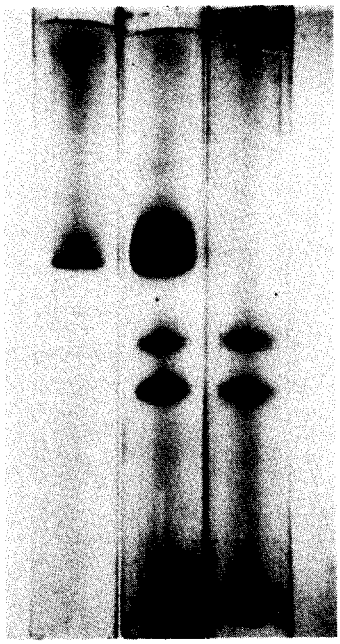

Fig.3. Polyacrylamide gel electrophoresis (10% polyacrylamide) in 6 M urea pH 3.2. Left, β-chain; middle, affinity purified pea-lectin; right, α-chain.

molecular species. The β-subunit migrated however as one band (fig.3). Similar results were obtained by Trowbridge [5] using isoelectric focusing. Although the charge heterogeneity of the α-chain would suggest the existence of isolectins, the N-terminal sequences of both α- and β-chains is unique.

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